Nitrogen Use Efficiency in Rice

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Rice (*Oryza sativa* L) is a daily staple food crop for more than half of the global population and improving productivity is an important task to meet future demands of the expanding world population. Application of nitrogen (N) fertilization improved rice growth and productivity in the world, but excess use causes environmental and economic issues. One of the main goals of rice breeding is reducing N fertilization while maintaining productivity. Therefore, enhancing rice nitrogen use efficiency (NUE) is essential for the development of sustainable agriculture and has become urgently needed. Many studies have been conducted on the main steps in the use of N including uptake and transport, reduction and assimilation, and translocation and remobilization, and on transcription factors regulating N metabolism. Understanding of these complex processes provides a base for the development of novel strategies to improve NUE for rice productivity under varying N conditions.

rice | nitrogen use efficiency | fertilization | productivity

1. Introduction

Rice is one of the major cereal grains to feed half of the world's population as a key nutritional source [1]. Accordingly, improving the production of rice is indispensable to satisfy the rising demands driven by population growth. The global population is expected to reach 10 billion, which will require a 70–100% increase in global crop production by 2050 [2]. The macronutrient N is an essential element for all living organisms because the synthesis of numerous vital biomolecules such as amino acids, proteins, nucleic acids, chlorophyll, and some plant hormones relies on N availability [3]. Thus, the application of N fertilizer has become a major factor responsible for the increase in crop yield over the past five decades, whereas excess usage of fertilization has caused serious environmental and economic issues [4]. The average nitrogen use efficiency (NUE) in crops is about 40–50% of the applied N, while the rest of the applied N is lost and enters the environments as N pollution [5]. The unutilized N causes water and air pollution affecting health, damage to biodiversity. The production of N fertilizer produced by the Haber–Bosch process requires a high amount of energy and contributes to greenhouse gas and then to climate change [6]. Besides, it represents increasing farmers' economic costs [7].

Improvement of NUE is one of the main objectives of breeding for rice that efficiently uptakes, assimilates, and remobilizes all available N resources [8]. Rice cultivars with high NUE are not only expected to increase grain yield but also to reduce environmental costs and facilitate low-input sustainable rice cultivation. Consequently, understanding the various processes of N metabolism is critical for increasing NUE.

2. N Assimilation and Reutilization

Assimilation of ammonium and utilization is a complex and tightly regulated process for rice biomass production and grain yield [9]. After nitrate is absorbed into roots, it is first reduced to nitrite by nitrate reductase (NR) in the cytoplasm, and then nitrite is further reduced to ammonium by nitrite reductase (NiR) in the plastids [10]. *OsNR2* encoding a NAD(P)H-dependent nitrate reductase (NR), was isolated as the master gene causing differences in nitrate assimilation and NUE between the *indica* and *japonica* rice [10]. Variations in *indica* and *japonica OsNR2* alleles result in structurally distinct OsNR2 proteins, with higher NR activity in *indica*, conferring increased effective tiller number, grain yield, and NUE compared to *japonica* rice [10]. The ammonium from nitrate reduction and ammonium uptake by OsAMTs is assimilated into amino acid via the glutamine synthetase (GS)/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle (Figure 1 and Table 2) [11].

2.1. Glutamine Synthetase

GS catalyzes an ATP-dependent conversion of glutamate (Glu) to glutamine (Gln) using ammonium and plays an essential role in the N metabolism. In rice, there are several homologous genes for the cytosolic GS (*OsGS1;1*, *OsGS1;2*, and *OsGS1;3*) and one chloroplastic gene (*OsGS2*) [12]. *OsGS1;1* is expressed in all organs and especially highly expressed in the leaf blade. Its disruption results in severe retardation of growth rate and lower grain productivity [12]. OsGS1;1 plays a critical role in maintaining the metabolic balance of rice plants grown under ammonium as an N source [12][13]. In addition, N assimilation by OsGS1;1 affects plastid development in rice roots [14]. The overexpression of *OsGS1;1* or *OsGS1;2* did not increase the grain yield or total amino acids in seeds [15].

OsGS1;2 is mainly expressed in the surface cells of roots that are responsible for the primary assimilation of ammonium [16]. Metabolic disorders caused by the lack of *GS1;2* lead to a severe reduction of the number of active tiller and grain yield [16][17][18]. *OsGS1;3* was exclusively expressed in the spikelet, but its functional role for N assimilation is still unknown [12]. OsGS2 isoform is abundant in the leaf and OsGS2 is primarily responsible for re-assimilation of ammonium produced from photorespiration in chloroplasts and assimilation in plastids of ammonium deriving from nitrate reduction [12][19]. The overexpression of *OsGS2* enhanced photoprotection and thereby enhanced tolerance to abiotic stresses [20].

2.2. Glutamate Synthetase

GOGAT yields two molecules of Glu by transferring the amine group of the amide side chain of Gln to 2-oxoglutarate (2-OG) [21]. Subsequently, one Glu molecule serves as a substrate for GS, while the other is used for transport, storage, or further amino acid metabolism. There are two types of GOGAT using either reduced ferredoxin (Fd-GOGAT) or NADH (NADH-GOGAT) as an electron donor. In rice, there are two NADH-type GOGATs and one Fd-GOGAT [22]. *OsNADH-OsGOAT1* is mainly expressed in the roots after the supply of ammonium [23], and *OsNADH-OsGOAT1* knockout mutant displays decreased grain yield in paddy field due to the reduced number of active tillers [22]. According to similarities in their expression patterns and the phenotypes of their knockout mutants, it was suggested that OsNADH-OsGOAT1 and OsGS1;2 both contribute to the primary assimilation of ammonium in rice roots [23]. Enhanced expression of *OsNADH-OsGOAT1* was shown to increase N uptake and N

remobilization efficiency, but the *OsNADH-OsGOAT1* overexpressing plants displayed poor growth benefits and reduced grain yield when grown in paddy field, which revealed some unbalanced use of N [24]. Nevertheless, the concomitant overexpression of both *OsNADH-OsGOAT1* and *OsAMT1;2* conferred enhanced NUE and better grain yield under N limiting growth conditions, which can provide a technical solution for plant performances under nitrate limiting conditions [24].

OsNADH-GOGAT2 is expressed mainly in fully expanded leaf blade and sheath. Defects in *OsNADH-GOGAT2* caused a remarkable reduction in spikelet number/panicle and grain yield and whole plant biomass [9]. OsNADH-GOGAT2 along with OsGS1;1 could be important in the remobilization of N during the senescence stage [23]. *OsFd-GOGAT* is mainly expressed in the chloroplasts of green tissues as well as the mature leaf blade and sheath and plays a major role in photorespiratory ammonium assimilation [25]. Mutation of *OsFd-GOGAT* led to premature leaf senescence and disturbed C/N balance [25][26].

2.3. Asparagine Synthetase, Glutamate Dehydrogenase, and Aspartate Aminotransferase

In addition to GS/GOGAT, several enzymes including asparagine synthetase (ASN), glutamate dehydrogenase (GDH), and aspartate aminotransferase (AAT) were characterized to play important roles in N metabolism (Figure 1 and Table 1) [6][27][28].

Asparagine (Asn) and Gln are the major N forms in phloem sap and play a critical role in dynamic N recycling [29] [30]. Asparagine synthetase (ASN) can produce asparagine by transferring the amide group from glutamine to aspartate. In *Arabidopsis*, labeling suggested that asparagine could be synthesized directly using ammonium as an N donor [31]. In rice, there are two *ASN* genes, *OsASN1* and *OsASN2* [32]. *OsASN1* is mainly expressed in roots in an ammonium-dependent manner, whereas *OsASN2* expression is decreased under ammonium supply [32]. Disruption of *OsASN1* leads to decreased N uptake and increased sensitivity to N limitation at the seedling stage. It displays reduced grain protein content and productivity, whereas the opposite tendencies are observed in overexpressing lines [33]. The mitochondrial NADH-dependent GDH catalyzes the reversible conversion of Glu to 2-OG and ammonia [34]. Three *OsNADH-GDH* genes are differentially expressed in various organs depending on N availability [35], but their roles remain unknown. AAT is involved in N and C metabolisms and catalyzes the reversible transamination of aspartate (Asp) to 2-OG, yielding Glu and oxaloacetate (OA) [28]. The overexpression of *OsAAT1* and *OsAAT2* resulted in altered N metabolism and increased amino acid content in seeds [28].

Table 1. Transporters and enzymes associated with N metabolic steps.

Gene Name	Locus Number	Phenotype Observed	References
OsNPF4.1/SP1	LOC_Os11g12740	Ko [1]: Defective in rice panicle elongation and the short-panicle phenotype	[36]
OsNPF8.9/OsNRT1.1	LOC_Os03g13274	OX [2]: Increased biomass under various N supplies	[37][38]

Gene Name	Locus Number	Phenotype Observed	References
		OX: Increased growth by N accumulation but decreased NUE [5] under high NH_4^+ supply	[39][40]
OsNPF7.3/OsPTR6	LOC_Os04g50950	OX: Enhanced NUE in paddy field	[41]
		RNAi [3]: Decreased amino acids accumulation and plant growth	
OsNPF8.20/OsPTR9	LOC_Os06g49250	OX: Enhanced N uptake, promotion of lateral root formation, and increased grain yield	[42]
		Ko & RNAi: The opposite effects of OX	
OsNPF2.2/OsPTR2	LOC_Os12g44100	Ko: Reduction in root-to-shoot nitrate transport and abnormal vasculature development	[43]
OsNPF2.4	LOC_Os03g48180	OX: Enhanced nitrate acquisition and upward transfer to shoot	[44]
		Ko: The opposite effects of OX	
OsNPF6.5/NRT1.1B	LOC_Os10g40600	NIL [4], OX: Increased grain yield and NUE	[45]
OsNPF7.2	LOC_Os02g47090	Ko & RNAi: Retarded growth under high nitrate supply	[46]
OsNPF8.1/OsPTR7	LOC_Os01g04950	Ko: Less accumulation of dimethyarsinate in rice grain	[47]
OsNPF7.7	LOC_Os10g42870	OX: Improved N influx in root, grain yield, and NUtE	[48]
OsNRT1.1A/OsNPF6.3	LOC_Os08g05910	OX: Early maturation and improved N utilization and grain yield	[49]
		Ko: Reduced N utilization and late flowering	
OsNPF6.1	LOC_Os01g01360	NIL: Enhancement of N uptake, NUE, and grain yield under low N supply	[50]
OsNRT2.3a	LOC_Os01g50820	RNAi: Defect in long-distance nitrate transport from root to shoot	[51]
OsNRT2.3b	LOC_Os01g50820	OX: Improved growth and NUE	[52]
OsNRT2.1	LOC_Os02g02170	OX: Fast growth under nitrate supply, but no increase in nitrate uptake	[53]
		OX by *OsNAR2.1* promoter: Increased grain yield and NUE	

Gene Name	Locus Number	Phenotype Observed	References
		OX: Increased grain yield and grain Mn under alternating wet and dry condition	[54]
		OX: Enhanced the nitrate-dependent root elongation	[55]
OsCLC1	LOC_Os01g65500	OX: Enhanced salt tolerance and grain yield	[56][57]
		Ko: The opposite effects of OX	
OsAMT1;1	LOC_Os04g43070	Ko: Decreased N uptake and the growth of roots and shoots	[58]
		OX: Improved NUE and grain yield	[59]
		OX: Decreased biomass at early stages of growth	[60]
OsAMT1;2	LOC_Os02g40730	Ac: Increased tolerance to N limitation at the seedling stage, but decreased grain yield	[24]
OsAMT1;3	LOC_Os02g40710	OX: Decreased growth with poor N uptake ability with a higher leaf C/N ratio	[61]
OsDUR3	LOC_Os10g42960	Ko: Decreased grain filling and grain yield	[62]
OsAAP6	LOC_Os01g65670	NIL, OX: Increased grain protein content	[63]
OsAAP3	LOC_Os06g36180	OX: Decrease in tiller number and grain yield	[64]
		Ko: Increased grain yield due to increased bud outgrowth and numbers of tillers	
OsAAP5	LOC_Os01g65660	OX: Less tiller number and grain yield	[65]
		RNAi: Increase in tiller number and grain yield	
OsAAP1	LOC_Os07g04180	OX: Increased, growth, tillering, and grain yield, higher concentrations of amino acids	[66]
		Ko: Inhibition of axillary bud outgrowth and reduced tiller number Ko & RNAi: The opposite effects of OX	
OsAAP4	LOC_Os12g09300	OX: Increased rice tillering and grain yield	[67]
OsLHT1	LOC_Os08g03350	Ko: Reduced amino acids uptake and allocation, growth inhibition, and low yield	[68][69][70]
OsNR2	LOC_Os02g53130	NIL: Increased effective tiller number, grain	[10]

Gene Name	Locus Number	Phenotype Observed	References
		yield, and NUE	
OsGS1;1	LOC_Os02g50240	Ko: Reduction in growth rate and grain yield	[12]
		Ko: Overaccumulation of free ammonium in the leaf sheath and roots	[13]
		Ko: Abnormal sugar and organic N accumulation	[14]
		OX: Decreases in grain yield and total amino acids in seeds	[15]
OsGS1;2	LOC_Os03g12290	OX: Reduction in grain yield and total amino acids in seeds	[15]
		Ko: Reduction in active tiller number and grain yield	[16][17][18]
OsGS2	LOC_Os04g56400	OX: Enhancement of photorespiration and tolerance to salt stress	[20]
OsNADH-GOGAT1	LOC_Os01g48960	Ko: Inhibition of the root elongation by NH_4^+, reduction in tiller number and grain yield	[22]
		Ac: Increased N uptake and remobilization, but a reduction in grain productivity	[24]
OsNADH-GOGAT2	LOC_Os05g48200	Ko: Reduction in Productivity Via Decrease of Spikelet Number	[9]
OsFd-GOGAT	LOC_Os07g46460	Ko: Premature leaf senescence and reduced grain yield with higher GPC	[25]
		Ko: Accumulation of an excessive amount of amino acids with disturbed C/N balance	[26]
OsASN1	LOC_Os03g18130	Ko: Reduction in free asparagine content in roots and xylem sap	[32]
		Ko: Decreased N uptake and grain yield	[33]
		OX: Enhanced tolerance and grain yield under N limitation	
OsAAT1	LOC_Os02g55420	OX: Increased amino acid content in seeds	[28]
OsAAT2	LOC_Os01g55540		

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